DEUTERATED CYCLOSPORINE ANALOGS AND THEIR USE AS IMMUNOMODULATING AGENTS

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ABSTRACT

Cyclosporine derivatives are disclosed which possess enhanced efficacy and reduced toxicity over naturally occurring and other presently known cyclosporins and cyclosporine derivatives. The cyclosporine derivatives of the present invention are produced by chemical and isotopic substitution of the cyclosporine A (CsA) molecule by: (1) Chemical substitution and optionally deuterium substitution of amino acid 1; and (2) deuterium substitution at key sites of metabolism of the cyclosporine A molecule such as amino acids 1, 4, 9. Also disclosed are methods of producing the cyclosporine derivatives and method of producing immunosuppression with reduced toxicity with the disclosed cyclosporine derivatives.
Fig. 1
Fig. 3
HO. AcO. MeLeu-MeVal - C - Abu-Sar MeLeu-MeVal st | Ac2O, Py | d)
MeLeu-D'Ala-Ala-MeLeu-Val-MeLeu

1

MeLeu-MeVal --N--C--Abu-Sar
MeLeu-D'Ala-Ala-MeLeu-Val-MeLeu

2

1) OsO4
2) NaIO4

MeLeu-MeVal --N--C--Abu-Sar
MeLeu-D'Ala-Ala-MeLeu-Val-MeLeu

3

1) (E)O2CHCH=PPh3Br
2) Acid hydrolysis

MeLeu-MeVal --N--C--Abu-Sar
MeLeu-D'Ala-Ala-MeLeu-Val-MeLeu

5

R = -D (5g)
- CO3 (5e)

MeLeu-MeVal --N--C--Abu-Sar
MeLeu-D'Ala-Ala-MeLeu-Val-MeLeu

6

1) RCD=PPh3PBr
2) K2CO3

Fig. 4
DEUTERATED CYCLOSPORINE ANALOGS AND THEIR USE AS IMMUNOMODULATING AGENTS

INTRODUCTION AND BACKGROUND

[0001] Cyclosporin derivatives of the present invention are disclosed which possess enhanced efficacy and reduced toxicity over naturally occurring and other presently known cyclosporins and cyclosporine derivatives. The cyclosporin derivatives of the present invention are produced by chemical and isotopic substitution of the cyclosporine A (CsA) molecule by:

[0002] 1. Chemical substitution and optionally deuterium substitution of amino acid 1; and

[0003] 2. Deuterium substitution at key sites of metabolism of the cyclosporine A molecule such as amino acids 1, 4, 9.

[0004] The cyclosporins are a family of, neutral, hydrophobic cyclic undecapeptides, containing a novel nine-carbon amino acid (McBmt) at position 1 of the ring that exhibit potent immunosuppressive, antiparasitic, fungidical, and chronic anti-inflammatory properties. The naturally occurring members of this family of structurally related compounds are produced by various fungi imperfecti. Cyclosporines A and C, are the major components. Cyclosporine A, which is discussed further below, is a particularly important member of the cyclosporin family of compounds. Twenty-four minor metabolites, also oligopeptides, have been identified: Lawen et al., J. Antibiotics 42, 1283 (1989); Traber et al., Helv. Chim. Acta 70, 13 (1987); Von Wartburg and Traber Prog. Med. Chem., 25, 1 (1988).


[0006] Cyclosporin was discovered to be immunosuppressive when it was observed to suppress antibody production in mice during the screening of fungal extracts. Specifically, its suppressive effects appear to be related to the inhibition of T-cell receptor-mediated activation events. It accomplishes this by interrupting calcium dependent signal transduction during T-cell activation by inactivating calmodulin and cyclophilin, a peptidyl prolyl cis-trans isomerase. It also inhibits lymphokine production by T-helper cells in vitro and arrests the development of mature CD8 and CD4 cells in the thymus. Other in vitro properties include inhibition of IL-2 producing T-lymphocytes and cytotoxic T-lymphocytes, inhibition of IL-2 released by activated T-cells, inhibition of resting T-lymphocytes in response to alloantigen and exogenous lymphokine, inhibition of IL-1 production, and inhibition of mitogen activation of IL-2 producing T-lymphocytes. Further evidence indicates that the above effects involve the T-lymphocytes at the activation and maturation stages.

[0007] Stimulation of TCR (T cell receptor) by foreign antigen on a major histocompatibility (MHC) molecule on the surface of the T cell results in the activation of a TCR signal transmission pathway (exact method of transmission unknown) through the cytoplasm causing the signal results in the activation of nuclear transcription factors, i.e. nuclear factors of activated T-cells (NF-AT) which regulate transcription of T-cell activation genes. These genes include that of lymphokine interleukin-2 (IL-2). Translation of the message is followed by secretion of IL-2. T-cell activation also involves the appearance of the lymphokine receptor IL-2R on the cell surface. After IL-2 binds to IL-2R, a lymphokine receptor (I. KR) signal transmission pathway is activated. The immunosuppressive drug, rapamycin, inhibits this pathway.

[0008] CsA is a potent inhibitor of TCR-mediated signal transduction pathway. It inhibits binding of NF-AT to the IL-2 enhancer, and thus inhibits transcriptional activation. CsA binds to cyclophilin, which binds to calcineurin, which is a key enzyme in the T-cell signal transduction cascade.

[0009] Cyclophilin is found in prokaryotic and eukarotic organisms and is ubiquitous and abundant. Cyclophilin is a single polypeptide chain with 165 amino acid residues. It has a molecular mass of 17.8 kD. A roughly spherical molecule with a radius of 17 angstroms, cyclophilin has a eight-stranded antiparallel beta barrel. Inside the barrel, the tightly packed core contains mostly hydrophobic side chains. CsA has numerous hydrophobic side chains which allow it to fit into the cyclophilin beta barrel. Cyclophilin catalyzes the interconversion of the cis and trans-rotamers of the peGlydyl-prolyl amide bond of peptide and protein substrates. Cyclophilin is identical in structure with peptidyl prolyl cis-trans isomerase and bears structural resemblance to the superfamily of proteins that transports ligands such as retinol-binding protein (RBP). These proteins carry the ligand in the barrel core. But cyclophilin actually carries the ligand binding site on the outside of the barrel. The tetrapeptide ligand binds in a long deep groove on the protein surface between one face of the beta barrel and the Thr116-Gly130 loop.


[0011] Mechanism of Cyclosporine A Action

[0012] Cyclosporine A-Cyclophilin A complex

[0013] CsA, as discussed above, binds to the cyclophilin beta barrel. Thirteen CyP residues define the CsA binding site. These residues are Arg 55, Phe 60, Met 61, Gln 63, Gly
The largest side-chain movements are 1.3 Å for Arg 55 and up to 0.7 Å for Phe 60, Gln 63, and Trp 121. There are four direct hydrogen bonds between the CyPA and CsA. Residues 4, 5, 6, 7, 8 of CsA protrude out into the solvent and are thought to be involved in binding the effector protein, calcineurin (Pflug, G., Kallen, J., Schirmer, T., Jansonius, J. N., Zurini, M. G. M., & Walkinshaw, M. D. (1993) Nature 361, 91-94.)


**[0017]** Metabolism of Cyclosporine:

**[0018]** Cyclosporine is metabolized in liver, small intestine and kidney to more than 30 metabolites. The structure of 13 metabolites and 2 phase II metabolites have been identified and at least 23 further metabolites have been isolated by HPLC and their structures characterized by mass spectrometry. The reactions involved in phase I metabolism of cyclosporine are hydroxylation, demethylation as well as oxidation and cyclization at amino acid 1. Several clinical studies and reports showed an association between blood concentrations of cyclosporine metabolites and neuro- or nephrotoxicity. In vitro experiments indicate that metabolites are considerably less immunosuppressive and more toxic than CsA.

**[0019]** As exemplified by the ever expanding list of indications for which CsA has been found useful, the cyclosporin family of compounds find utility in the prevention of rejection or organ and bone marrow transplants, and in the treatment of psoriasis, and a number of autoimmune disorders such as type 1 diabetes mellitus, multiple sclerosis, auto immune uveitis, and rheumatoid arthritis. Additional indications are discussed infra.

**[0020]** As is generally accepted by those of skill in the art, inhibition of secretion of interleukin-2 (IL-2) and other lymphokines from lymphocytes, is a useful indicator of intrinsic immunosuppressive activity of a cyclosporin analog. For a recent review of cyclosporin uses and mechanisms of action see Wenger et al Cyclosporine: Chemistry, Structure-Activity Relationships and Mode of Action, Progress in Clinical Biochemistry and Medicine, vol. 2, 176 (1986).

**[0021]** Cyclosporin A is a cyclic peptide which contains several N-methyl amino acids and, at position-8, contains a D-alanine. The structure of Cyclosporin A is given below:

![Cyclosporin A structure](image)

*Unless otherwise specified, each of the amino acids of the disclosed cyclosporin is of the L-configuration.

**[0022]** As is the practice, in the field, a particular cyclosporin analog may be named using a shorthand notation identifying how the analog differs from cyclosporin A. Thus, cyclosporin C which differs from cyclosporin A by the threonine at position-2 may be identified as [Thr]²-cyclosporin or [Thr]²-CsA. Similarly, cyclosporin B is [Ala]
cyclosporin D is [Val]²-CsA; cyclosporin E is [Val]¹¹-CsA; cyclosporin F is [3-DesoxyMeBmt]¹²-CsA; cyclosporin G is [NVa]³³-CsA; and cyclosporin H is [D-MeVa]¹³-CsA.

D-Serine and D-Threonine have been introduced into the 8-position of cyclosporin A by biosynthesis resulting in active compounds. See R. Traber et al. J. Antibiotics 42, 591 (1989). D-Chloroalanine has also been introduced into position-8 of Cyclosporin A by biosynthesis. See A. Lawen and J. Antibiotics 5-2, 1283 (1989).

Indications for Cyclosporine Therapy

Immune-regulatory abnormalities have been shown to exist in a wide variety of autoimmune and chronic inflammatory diseases, including systemic lupus erythematosus, chronic rheumatoid arthritis, type 1 diabetes mellitus, inflammatory bowel disease, bilary cirrhosis, uveitis, multiple sclerosis and other disorders such as Crohn's disease, ulcerative colitis, bullous pemphigoid, sarcoidosis, psoriasis, ichthyosis, and Graves ophthalmopathy. Although the underlying pathogenesis of each of these conditions may be quite different, they have in common the appearance of a variety of autoantibodies and self-reactive lymphocytes. Such self-reactivity may be due, in part, to a loss of the homeostatic controls under which the normal immune system operates.

Similarly, following a bone marrow or an organ transplantation, the host lymphocytes recognize the foreign tissue antigens and begin to produce antibodies which lead to graft rejection.

One end result of an autoimmune or a rejection process is tissue destruction caused by inflammatory cells and the mediators they release. Anti-inflammatory agents, such as NSAID's (Non-Steroidal Anti-inflammatory Drugs), and corticosteroids act principally by blocking the effect of, or secretion of, these mediators, but do nothing to modify the immunologic basis of the disease. On the other hand, cytotoxic agents, such as cyclophosphamide, act in such a nonspecific fashion that both the normal and autoimmune responses are shut off. Indeed, patients treated with such nonspecific immunosuppressive agents are at risk to succumb to infection as they are to their autoimmune disease.

Generally, a cyclosporin, such as cyclosporine A, is not cytotoxic nor myelotoxic. It does not inhibit migration of monocytes nor does it inhibit granulocytes and macrophage action. Its action is specific and leaves most established immune responses intact. However, it is nephrotoxic and is known to cause the following undesirable side effects:

1. abnormal liver function;
2. hirsutism;
3. gum hypertrophy;
4. tremor;
5. neurotoxicity;
6. hyperaesthesia; and
7. gastrointestinal discomfort.

A number of cyclosporines and analogs have been described in the patent literature.


U.S. Pat. No. 4,210,581 issued to Ruegger, et al. on Jul. 1, 1980 entitled, "Organic Compounds", discloses cyclosporin C and dihydrocyclosporin C which can be produced by hydrogenation of cyclosporin C.


U.S. Pat. No. 4,384,996, issued to Bollinger, et al. on May 24, 1983 entitled "Novel Cyclosporins", discloses cyclosporins having a β-vinylene-ε- amino acid residue at the 2-position and/or a β-hydroxy-ε-amino acid residue at the 8-position. The cyclosporins disclosed included either McBmt or dihydro-McBmt at the 1-position.

U.S. Pat. No. 4,396,542, issued to Wenger on Aug. 2, 1983 entitled, "Method for the Total Synthesis of Cyclosporins, Novel Cyclosporins and Novel Intermediates and Methods for their Production", discloses the synthesis of cyclosporins, wherein the residue at the 1-position is either McBmt, dihydro-McBmt, and protected intermediates.


U.S. Pat. No. 4,703,033, issued to Seebach on Oct. 27, 1987 entitled, "Novel Cyclosporins", discloses cyclosporins with substituted residues at positions 1, 2 and 3. The substitutions at position-3 include halogens.


Additional cyclosporin analogs are disclosed in U.S. Pat. No. 4,798,823, issued to Witzel, entitled, New
Cyclosporin Analogs with Modified “C-9 amino acids”, which discloses cyclosporin analogs with sulfur-containing amino acids at position-1.

SUMMARY OF THE INVENTION

[0050] The present invention concerns chemically substituted and deuterated analogs of cyclosporine A and related cyclosporines.

[0051] An object of the present invention is to provide new cyclosporine analogs which have enhanced efficacy and altered pharmacokinetic and pharmacodynamic parameters. Another object of the present invention is to provide a cyclosporine analog for the care of immunoregulatory disorders and diseases, including the prevention, control and treatment thereof. An additional object of the present invention is to provide pharmaceutical compositions for administering to a patient in need of the treatment one or more of the active immunosuppressive agents of the present invention. Still a further object of this invention is to provide a method of controlling graft rejection, autoimmune and chronic inflammatory diseases by administering a sufficient amount of one or more of the novel immunosuppressive agents in a mammalian species in need of such treatment. Finally, it is the object of this invention to provide processes for the preparation of the active compounds of the present invention.

[0052] Substitution and deuteration of the cyclosporine molecule results in altered physiochemical and pharmacokinetic properties which enhance its usefulness in the treatment of transplantation rejection, host vs. graft disease, graft vs. host disease, aplastic anemia, focal and segmental glomerulosclerosis, myasthenia gravis, psoriatic arthritis, relapsing polyarthritis and ulcerative colitis.

[0053] Embodiments of the invention include CsA derivatives wherein one or more hydrogen atoms in the 1, 3 and 9 amino acid positions can be substituted with a deuterium atom and wherein the cyclosporine A derivatives are option-ally chemically substituted at the amino acid 9 position. A further specific embodiment of the invention is the CsA derivative represented by formula (I):

[0054] where R is (i) a deuterium or (ii) a saturated or unsaturated straight or branched aliphatic chain of from 2 to 16 carbon atoms and optionally containing one or more deuterium atoms or an ester, ketone or alcohol of the carbon chain and optionally containing one or more substituents selected from halogen, nitro, amino, amido, aromatic, and heterocyclic, or (iii) R is an aromatic or heterocyclic group optionally containing a deuterium atom, or (iv) R is a methyl group and X, Y, and Z are hydrogen or deuterium provided that at least one of X, Y or Z is deuterium and R' is an OH or an ester or is an O and together with a carbon adjacent to a double bond on amino acid 1 form a heterocyclic ring such as 5-membered rings where the heteroatom is oxygen. Other specific embodiments of the present invention include the CsA derivative of formula (I) where R is a saturated or unsaturated carbon chain of from 2 to 3 carbons containing one or more deuterium. Further specific embodiments include those of formulas 5g and 5e below:

\[
\begin{align*}
\text{(5g)} & \quad \text{CD}2 \\
\text{MeLeu} & \quad \text{MeVal} - \text{N} \quad \text{Abu} - \text{Sar} \\
\text{MeLeu} & \quad \text{D-Ala} - \text{MeLeu} \quad \text{Val} - \text{MeLeu}
\end{align*}
\]
DESCRIPTION OF THE FIGURES

[0055] FIG. 1 is the structure of cyclosporine A showing a site of deuteration at the amino acid 3 position.

[0056] FIG. 2 is the structure of cyclosporine A showing a site of deuteration at the amino acid 9 position.

[0057] FIG. 3 is scheme I of the synthesis of the cyclosporine derivatives.

[0058] FIG. 4 is scheme II of the synthesis of the cyclosporine derivatives.

DETAILED DESCRIPTION OF THE INVENTION

[0059] Substitution of deuterium for ordinary hydrogen and deuterated substrates for protio metabolites can produce profound changes in biosystems. Isotopically altered drugs have shown widely divergent pharmacological effects. Petersen et al., found increased anti-cancer effect with deuterated 5,6-benzylidine-dl-L-ascorbic acid (Zilascorb) [Anticancer Res. 12, 33 (1992)].

[0060] Substitution of deuterium in methyl groups of cyclosporine will result in a slower rate of oxidation of the C13 D bond relative to the rate of oxidation of a non-deuterium substituted C—H bond. The isotopic effect acts to reduce formation of demethylated metabolites and thereby alters the pharmacokinetic parameters of the drug. Lower rates of oxidation, metabolism and clearance result in greater and more sustained biological activity. Deuteration is targeted at various sites of the cyclosporin molecule to increase the potency of drug, reduce toxicity of the drug, reduce the clearance of the pharmacologically active moiety and improve the stability of the molecule.

[0061] Isotopic Substitution:

[0062] Stable isotopes (e.g., deuterium, $^{13}$C, $^{15}$N, $^{18}$O) are nonradioactive isotopes which contain one additional neutron than the normally abundant isotope of the respective atom. Deuterated compounds have been used in pharmaceutical research to investigate the in vivo metabolic fate of the compounds by evaluation of the mechanism of action and metabolic pathway of the non deuterated parent compound. (Blake et al. J. Pharm. Sci. 64, 3, 367-391, 1975). Such metabolic studies are important in the design of safe, effective therapeutic drugs, either because the in vivo active compound administered to the patient or because the metabolites produced from the parent compound prove to be toxic or carcinogenic (Foster et al., Advances in Drug Research Vol. 14, pp. 2-36, Academic press, London, 1985).

[0063] Incorporation of a heavy atom particularly substitution of deuterium for hydrogen, can give rise to an isotope effect that could alter the pharmacokinetics of the drug. This effect is usually insignificant if the label is placed at a metabolically inert position of the molecule.

[0064] Stable isotope labeling of a drug can alter its physico-chemical properties such as pKa and lipid solubility. These changes may influence the fate of the drug at different steps along its passage through the body. Absorption, distribution, metabolism or excretion can be changed. Absorption and distribution are processes that depend primarily on the molecular size and the lipophilicity of the substance. These effects and alterations can affect the pharmacodynamic response of the drug molecule if the isotopic substitution affects a region involved in a ligand-receptor interaction.

[0065] Drug metabolism can give rise to large isotopic effect if the breaking of a chemical bond to a deuterium atom is the rate limiting step in the process. While some of the physical properties of a stable isotope-labeled molecule are different from those of the unlabeled one, the chemical and biological properties are the same, with one important exception: because of the increased mass of the heavy isotope, any bond involving the heavy isotope and another atom will be stronger than the same bond between the light isotope and that atom. In any reaction in which the breaking of this bond is the rate limiting step, the reaction will proceed slower for the molecule with the heavy isotope due to "kinetic isotope effect". A reaction involving breaking a C13D bond can be up to 700 per cent slower than a similar reaction involving breaking a C—H bond. If the C13D bond is not involved in any of the steps leading to the metabolite, there may not be any effect to alter the behavior of the drug. If a deuterium is placed at a site involved in the metabolism of a drug, an isotope effect will be observed only if breaking of the C13D bond is the rate limiting step. There is evidence to suggest that whenever cleavage of an aliphatic C—H bond occurs, usually by oxidation catalyzed by a mixed-function oxidase, replacement of the hydrogen by deuterium will lead to observable isotope effect. It is also important to understand that the incorporation of deuterium at the site of metabolism slows its rate to the point where another metabolite produced by attack at a carbon atom not substituted by deuterium becomes the major pathway a process called "metabolic switching". It is also observed that one of the most important metabolic pathways of compounds containing aromatic systems is hydroxylation leading to a phenolic group in the 3 or 4 position to carbon substituents. Although this pathway involves cleavage of the C—H bond, it is often not accompanied by an isotope effect, because the cleavage of this bond mostly not involved in the rate limiting step. The substitution of hydrogen by deuterium at the stereo center will induce a greater effect on the activity of the drug.

[0066] Synthesis of Cyclosporine Derivatives:

[0067] The starting material for the preparation of the compounds of this invention is cyclosporine A. The process for preparing the compounds of the present invention are
illustrated as shown in scheme 1 in FIG. 3. It will be readily apparent to one of ordinary skill in the art reviewing the synthetic route depicted below that other compounds with formula I can be synthesized by substitution of appropriate reactants and agents in the synthesis shown below.

[0068] The first step in the process for making deuterated cyclosporin analogs is the preparation of the key intermediate 3 and 6. This can be achieved by the oxidation of the double bond in the amino acid 1. Treatment of cyclosporin with acetic anhydride and excess of dimethylaminopyridine provided the hydroxyl protected acetyl cyclosporin 2. Although cleavage of the double bond could then be accomplished by treatment of 2 with ozone, or KMnO₄/NaIO₄, it was found out that OsO₄/NaIO₄ was the reagent of choice for the transformation to the aldehyde product 3. The reaction was generally found to be cleaner, producing the required material and to proceed in higher yield. The drawback to this reaction is that OsO₄ is expensive and highly toxic, so that its use is limited. But the results can be accomplished more economically by the use of H₂O₂ with OsO₄ present in catalytic amounts. tert-butyl hydroxide in alkaline solution and N-methylmorpholine-N-oxide can be substituted for H₂O₂ in this process. The aldehyde compound 3 was further treated with various deuterated alkyl or aryl triphenyl phosphonium derivatives with reagents and hydrolysis by alkaline solution provided the final derivatives (5 a-h). We also developed a general procedure to obtain various compounds as shown in Scheme II in FIG. 4.

[0069] In this approach, the aldehyde derivative 3 was treated with the Wittig reagent prepared by using standard procedure. The resultant product on mild acid hydrolysis provided the key intermediate aldehyde product 6. This was further treated with second deuterated alkyl or aryl triphenyl phosphonium halide reagents and on mild acid hydrolysis yielded the required products. This method provides control over the extension of the diene system. By using this approach, olefinic double bonds can be introduced step by step.

[0070] A third approach to prepare the deuterated compounds 5a-h—is by heating non deuterated cyclosporin analogs described earlier, in a deuterated solvent such as deuterated water, deuterated acetic acid in the presence of acid or base catalyst.

[0071] Preferred cyclosporins of the present invention include those which contain a deuterium and/or a chemical substitution on amino acid 1 such as those of formula II:

\[
\text{[0072]} \quad \text{And } R= -\text{CH}_2, -\text{CHO}, -\text{CD}, -\text{CH} = \text{CD}, -\text{CD} = \text{CD}, -\text{CH} = \text{CH} = \text{CD}, -\text{CD} = \text{CD} = \text{CD}, -\text{CH} = \text{CH} = \text{CH} = \text{CD}, -\text{CH} = \text{CH} = \text{CD}, -\text{CH} = \text{CD}, -\text{CH} = \text{CH}.
\]

EXAMPLES

Example 1

[0073] To a stirred solution of cyclosporine 1 (101.4 g, 0.84 mmol) in acetic anhydride (20 mL) at room temperature was added DMAP (150 mg, 1.23 mmol 1.5 eq). After stirring overnight, the reaction mixture was partitioned between EtOAc (50 ml) and water (25 ml). The separated EtOAc layer was then washed with water (50 ml) and brine (50 ml), dried (MgSO₄) and the solvent removed in vacuo to give the crude product as a glassy solid. Purification by flash chromatography through a short column of silica (2% MeOH/DCM) and lyophilisation from benzene yielded 2 (104.2 g, 0.84 mmol, quant.) as a fluffy, colourless solid; \(\delta_{1H}^{}=305.7\) (c= 0.3, CHCl₃); \(\nu_{max}\) (CHCl₃ cast) cm⁻¹: 3328m, 2963m, 1746m, 1627s, 1528m, 1472m, 1233m; \(\delta_{1H}\) (600 MHz, CD₂SO) 8.73 (1H, d, J=9.5 Hz, NH), 8.30 (1H, d, J=7.0 Hz, NH), 7.92 (1H, d, J=7.5 Hz, NH), 7.49 (1H, d, J=7.5 Hz, NH), 6.50 (1H, d, J=11.5 Hz), 5.88 (1H, dd, J=3.5, 11.5 Hz), 5.82 (1H, d, J=11.5 Hz), 5.65 (1H, dd, J=4.0, 12.0 Hz), 5.60 (1H, dd, J=3.5, 12.5 Hz), 5.63-5.57 (1H, m), 5.51-5.45 (1H, m), 5.37 (1H, dd, J=5.5, 8.5 Hz), 5.05-5.01 (2H, complex), 4.99 (1H, d, J=11.0 Hz), 4.76 (1H, p, J=7.0 Hz), 4.58 (1H, p, J=7.0 Hz), 4.02 (1H, d, J=13.5 Hz), 3.47 (3H, s), 3.30 (3H, s), 3.17 (3H, s), 3.11 (3H, s), 2.98 (3H, s), 2.68-2.62 (1H, m), 2.63 (3H, s), 2.51-2.39 (2H, complex), 2.34-2.25 (8H, complex), 2.05 (3H, s), 1.97-1.85 (2H, complex), 1.83 (3H, dd, J=1.0, 6.5 Hz), 1.82-1.77 (2H, complex), 1.68-1.61 (3H, complex), 1.55 (3H, dd, J=7.0 Hz), 1.55-1.51 (1H, m), 1.44-1.38 (1H, m), 1.32-1.20 (2H, complex), 1.29 (3H, d, J=6.5 Hz), 1.17

where X is

\[
\begin{array}{ccccccccccc}
\text{X} & \text{Abu} & \text{Sa} & \text{MeLeu} & \text{Va} & \text{MeLeu} & \text{Ala} & \text{(D)Ala} & \text{MeLeu} & \text{MeLeu} & \text{MeVal} \\
\text{1} & \text{2} & \text{3} & \text{4} & \text{5} & \text{6} & \text{7} & \text{8} & \text{9} & \text{10} & \text{11}
\end{array}
\]

Where X is
(3H, d, J=6.5 Hz), 1.14 (3H, d, J=6.5 Hz), 1.08 (3H, d, J=6.5 Hz), 1.04 (3H, d, J=6.0 Hz), 1.03 (3H, d, J=7.0 Hz), 1.00 (3H, d, J=7.0 Hz), 0.93 (3H, d, J=6.0 Hz), 0.92 (3H, d, J=6.5 Hz), 0.88-0.84 (9H, complex), 0.76 (3H, d, J=6.5 Hz), 0.57 (3H, d, J=6.5 Hz); δC (75 MHz, C_D) 173.6, 173.2, 172.8, 172.6, 171.3, 171.1, 207.0, 170.4, 170.2, 169.8, 167.9 (C=O), 129.0, 126.2 (C=C), 73.1 (COAc), 58.1, 57.1, 56.0, 55.0, 54.6, 54.2, 50.3, 49.9, 48.6, 48.1, 47.8, 44.5, 40.8, 39.1, 35.7, 33.6, 32.9, 32.1, 31.5, 31.2, 30.0, 29.7, 29.5, 23.4, 24.6, 24.4, 23.4, 23.3, 21.7, 21.1, 21.0, 20.6, 20.3, 19.5, 18.5, 18.0, 17.7, 17.5, 17.4, 14.9, 9.7; m/z (Electrospray)

Example 2

To a solution of compound 2 (289 mg, 0.23 mmol) in a 1:1 mixture of dioxane and water (5 mL) was added firstly sodium metaperiodate (100 mg, 0.47 mmol, 2 eq) and subsequently a solution of osmium tetroxide (0.5 mL, 0.5 g OsO4, in 250 mL of solvent). Two-phase work-up, purification by flash column chromatography (40% acetone in petroleum ether) and lyophilisation from benzene yielded compound 3. (226 mg, 0.18 mmol, 80%) as a flaky, colourless solid; [α]D 25 ±2600 (c. 0.1, CHCl3); νmax (CHCl3 cast)-cm⁻¹ 3325m, 2962m, 1748w, 1724w, 1677m, 1626s, 1282m, 755s; δH (300 MHz, CDCl3) 8.63 (1H, d, J=9.5 Hz, NH), 8.16 (1H, d, J=7.0 Hz, NH), 7.95 (1H, d, J=7.5 Hz, NH), 7.48 (1H, d, J=9.0 Hz, NH), 5.93 (1H, d, J=7.5 Hz), 5.84 (1H, d, J=4.0 Hz), 5.70 (1H, d, J=1.5 Hz), 5.56-5.54 (1H, m), 5.32 (1H, dd, J=5.5, 8.0 Hz), 5.07-4.88 (CH, complex), 4.72 (1H, p, J=7.0 Hz), 4.49 (1H, p, J=7.0 Hz), 3.98 (1H, d, J=14.0 Hz), 3.42 (3H, s, CH3), 3.27 (3H, s, CH3), 3.12 (3H, s, CH3), 3.07 (3H, s, CH3), 2.91 (3H, s, CH3), 2.79 (3H, s, CH3), 2.59 (3H, s, CH3), 2.42-2.08 (10H, complex), 1.94 (3H, s, CH3CO), 1.47 (3H, d, J=7.0 Hz), 1.24 (3H, 7.0 Hz), 1.14-1.09 (9H, complex), 1.04 (3H, d, J=6.5 Hz), 1.01 (3H, d, J=7.0 Hz), 0.96 (3H, d, J=6.5 Hz), 0.92 (3H, d, J=6.5 Hz), 0.91 (3H, d, J=6.5 Hz), 0.89 (3H, d, J=6.0 Hz), 0.83 (6H, d, J=6.5 Hz), 0.74 (3H, d, J=6.5 Hz), 0.59 (3H, d, J=6.5 Hz), δ (75 MHz, C_D) 202.5 (CHO), 174.6, 174.0, 173.7, 172.8, 172.5, 171.5, 171.2, 171.1, 170.6, 170.2, 170.2, 168.0, 73.0, 58.7, 57.6, 56.7, 55.5, 55.0, 54.5, 49.4, 48.9, 48.5, 48.1, 45.0, 44.6, 43.1, 39.8, 38.8, 37.7, 36.2, 32.5, 32.0, 31.6, 30.9, 30.3, 29.8, 29.6, 29.6, 25.6, 25.3, 25.0, 24.8, 24.5, 24.0, 23.8, 22.0, 21.7, 21.2, 20.5, 20.0, 19.8, 18.8, 18.5, 18.2, 17.4, 15.2, 10.0; m/z (Electrospray) 1232.8 (MH⁺, 100%).

Example 4

To a stirred solution of 4d (84 mg, 67 µmol) in MeOH (5 mL) and water (2.5 mL) at room temperature was added potassium carbonate (99 mg, 0.72 mmol, -10 eq). After stirring overnight, the MeOH was removed in vacuo and the aqueous residue was partitioned between EtOAc (10 mL) and 5% citric acid solution (10 mL). The EtOAc layer was then washed with water (10 mL) and brine (10 mL), dried (MgSO4) and the solvent removed in vacuo to give the crude product. HPLC purification (60% to 65% MeCN in water) and lyophilisation from benzene yielded compound 3d (35 mg, 49 µmol, 70%) as a flaky, colourless solid; [α]D 25 ±262.0 (c. 0.05, CHCl3); νmax (CHCl3 cast)-cm⁻¹ 3318m, 3008s, 2960s, 2872m, 1627s, 1519m, 1470m, 1411m, 1295m, 1055m, 754s, δD (600 MHz, CDCl3) 8.27 (1H, d, J=9.5 Hz), 7.96 (1H, d, J=7.5 Hz), 7.63 (1H, d, J=8.0 Hz), 7.45 (1H, d, J=9.0 Hz), 5.87 (1H, d, J=5.5, 11.0 Hz), 5.74 (1H, d, J=7.5 Hz), 5.72-5.69 (1H, m), 5.66-5.64 (1H, br d, J=11.0 Hz), 5.79 (1H, dd, J=4.0, 11.5 Hz), 3.39 (1H, dd, J=5.5, 10.5 Hz), 5.33 (1H, dd, J=5.5, 8.5 Hz), 5.24 (1H, d, J=11.0 Hz), 5.12 (1H, dt, J=7.5, 10.0 Hz), 4.88-4.79 (3H, complex), 4.22 (1H, dd, J=5.5, 7.5 Hz, 4.00 (1H, d, J=13.5 Hz), 3.72 (3H, s), 3.22 (3H, s), 3.06 (3H, s), 2.97 (3H, s), 2.92 (3H, s), 2.85 (3H, s), 2.67-2.60 (1H, m), 2.58 (3H, s), 2.56-2.50 (1H, br m), 2.33-2.23 (4H, complex), 2.20-2.07 (4H, complex), 1.80-1.74 (3H, complex), 1.67 (3H, d, J=7.0 Hz), 1.50-1.50 (2H, complex), 1.46-1.25 (9H, complex), 1.17-1.13 (16H, complex), 1.06 (3H, d, J=6.5 Hz), 1.02 (3H, d, J=7.0 Hz), 0.98 (3H, d, J=6.5 Hz), 0.96 (3H, d, J=7.0 Hz), 0.92-0.89 (9H complex), 0.86 (3H, t, J=7.5 Hz), 0.83 (3H, d, J=6.5 Hz), 0.78 (3H, d, J=6.0 Hz), 0.75 (3H, d, J=7.0 Hz), 0.73 (3H, d, J=6.5 Hz), 0.71-0.69 (6H complex) and 0.64-0.60 (9H, complex).
Example 5

**[0078]** To a vigorously stirred mixture of compound 3 (49 mg, 39.81 µmol) and deuterated d₆-alllyltriphenylphosphonium bromide (311 mg, 812 µmol, −20 °C) in benzene (3 mL) at room temperature was added 1N NaOH (3 mL). Stirring was continued at room temperature for 5 days, after which the 2 layers were separated. The benzene layer was washed with water (5 mL), dried (MgSO₄) and the solvent removed in vacuo to give the crude product. Purification by HPLC (20% to 60% MeCN in water) and lyophilisation from benzene yielded compound 4 (25 mg, 18.3 µmol, 47%) as a white solid. 1H NMR (400 MHz, CDCl₃) δ 7.5-7.55, 5.5-5.54, 4.9-4.90, 4.8-4.85, 4.53-4.52, 4.3-4.14, 3.96-3.90, 3.78, 3.65, 3.61, 3.58, 3.37-3.16, 3.08, 3.04, 3.01, 2.99, 2.95, 2.94, 2.55, 2.52, 2.50, 2.49, 2.45, 2.42, 2.38, 2.37, 2.36, 2.20, 2.14, 20.0, 18.8, 18.5, 17.8, 16.0, 10.1 ppm; m/z (Electrospray) 1206 ([M+H]+, 30%), 1228 ([M+Na]+, 100), 1244 ([M+K]+, 25).

Example 7

**[0080]** To a stirred solution of compound 4g (20 mg, 15.9 µmol) in methanol (5 mL) and water (1 mL) at room temperature was added potassium carbonate (30 mg, 217 µmol). After stirring overnight, the reaction mixture was partitioned between EtOAc (10 mL) and 5% aqueous citric acid (10 mL). The aqueous layer was further extracted with EtOAc (5 mL), the combined organic layers were then washed with 5% citric acid (10 mL) and brine (10 mL), dried (MgSO₄) and the solvent removed in vacuo to give the crude product. Purification by HPLC (65% MeCN) and lyophilisation from benzene yielded compound 5g (10 mg, 8.2 µmol, 52%) as a fluffy, colourless solid; [α]D²⁵ = −28.2 (c. 0.29, CHCl₃), λmax (CHCl₃) cm⁻¹ 3500-3200, 3319m, 2958m, 2927m, 1626s, 1520m, 1468s, 754m; δ(CDCl₃, C₆D₆) complex due to the presence of 4 isomers 8.76 (d, J=6.0 Hz), 8.73 (d, J=6.0 Hz), 8.29 (d, J=7.0 Hz), 7.93 (d, J=7.5 Hz), 7.88 (d, J=7.5 Hz), 7.53 (d, J=5.5 Hz), 7.62-7.31 (1H, complex), 7.16-6.88 (2H, complex), 6.58-6.39 (complex), 6.26 (d, J=11.0 Hz), 6.15 (d, J=10.5 Hz), 6.09 (d, J=10.5 Hz), 6.05 (d, J=11.5 Hz), 5.90-5.82 (complex), 5.68-5.35 (complex), 5.08-4.97 (complex), 4.81-4.72 (complex), 4.63-4.53 (complex), 4.03 (d, J=14.0 Hz), 3.47 (s), 3.46 (s), 3.28 (s), 3.26 (s), 3.17 (s), 3.15 (s), 3.09 (s), 2.98 (s), 2.97 (s), 2.83 (s), 2.63 (s), 2.62 (s), 2.71-2.56 (complex), 2.47-2.23 (complex), 2.05 (t), 2.04 (s), 2.03 (s), 2.02 (s), 1.98-0.82 (complex), 0.77 (d, J=6.5 Hz), 0.58 (d, J=6.5 Hz), 0.58 (t, J=6.5 Hz), m/z (Electrospray) 1273.8 (M⁺, 100%).

Example 8

**[0081]** To a stirred solution of compound 4e (18 mg, 14.2 µmol) in methanol (5 mL) and water (1 mL) at room temperature was added potassium carbonate (35 mg, 254 µmol). After stirring overnight, the reaction mixture was partitioned between EtOAc (10 mL) and 5% aqueous citric acid (10 mL). The aqueous layer was further extracted with EtOAc (5 mL), the combined organic layers were then washed with 5% citric acid (10 mL) and brine (10 mL), dried (MgSO₄) and the solvent removed in vacuo to give the crude product. Purification by HPLC (65% MeCN) and lyophilisation from benzene yielded compound 5e (10 mg, 8.1 µmol, 57%) as a fluffy, colourless solid; [α]D²⁵ = −285.5 (c. 0.11, CHCl₃), δ(CDCl₃) cm⁻¹ 3000 MHz, C₆D₆) complex due to the presence of 4 isomers 8.31 (d, J=9.5 Hz), 8.28 (d, J=9.5 Hz), 8.16 (d, J=9.5 Hz), 8.14 (d, J=10.5 Hz), 8.14 (d, J=7.5 Hz), 7.96 (d, J=7.5 Hz), 7.95 (d, J=7.0 Hz), 7.86 (d, J=7.7 Hz), 7.85 (d, J=7.5 Hz), 7.63 (d, J=7.5 Hz), 7.59 (d, J=7.5 Hz), 7.50-7.44 (complex), 6.60-6.49 (complex), 6.36-6.11 (complex), 5.88-5.83 (complex), 5.76-
Example 9

The immnosuppressive activity can be tested for cyclosporine and the disclosed cyclosporine analogs as described below. Calcineurin activity is assayed using a modification of the method previously described by Fruman et al. (A Proc Natl Acad Sci USA, 1992). Whole blood lysates are evaluated for their ability to dephosphorylate a 32P-labelled 19 amino acid peptide substrate in the presence of okadaic acid, a phosphatase type 1 and 2 inhibitor. Background phosphatase 2C activity (CsaA and okadaic acid resistant activity) is determined and subtracted from each sample, with the assay performed in the presence and absence of excess added CsaA. The remaining phosphatase activity is taken as calcineurin activity.

Example 10

A mixed lymphocyte reaction (MLR) assay is performed with cyclosporine and the disclosed cyclosporine analogs. The MLR assay is useful for identifying CSA derivatives with biological (immunosuppressive) activity and to quantify this activity relative to the immunosuppressive activity of the parent CSA molecule.

Example 11

Other cyclosporine derivatives of the invention which have been prepared include the following:

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5.71 (complex), 5.64-5.22 (complex), 5.17-5.08 (complex), 4.91-4.77 (complex), 4.26-4.18 (complex), 3.99 (d, J=14.0 Hz), 3.97 (d, J=14.0 Hz), 3.74 (s), 3.73 (s), 3.71 (s), 3.69 (s), 3.22 (s), 3.21 (s), 3.20 (s), 3.19 (s), 3.07 (s), 3.06 (s), 3.05 (s), 2.97 (s), 2.96 (s), 2.95 (s), 2.92 (s), 2.91 (s), 2.89 (s), 2.84 (s), 2.83 (s), 2.69-2.07 (complex), 2.58 (s), 2.57 (s), 1.84-0.81 (complex), 0.64 (d, J=6.5 Hz); m/z (Electrospray) 1269.8 ([M+K]+, 5%), 1253.8 ([M+Na]+, 30), 1231.8 (MH+).

Aliquot 100 μl per well lymphocyte population A

Aliquot 20 μl per well of drug (CSA and CSA derivatives) at 0, 2.5, 5, 10, 25, 50 and 100 μg/L in triplicate in DMEM with no supplements.

To measure the effect of drug on proliferation, incubate the plate for 5 days at 37°C in 5% CO2 atmosphere.

On day 6, prepare 32 mls of 1:50 dilution of Methyl-1H-Thymidine (Amersham Life Science, cat # TRK 120) in DMEM with no supplements. Add 30 μl per well and incubate for 18 hours at 37°C in 5% CO2 atmosphere.

10. On day 7 cells are harvested onto glass microfiber filters GF/A (Whatman, cat # 1820024) using a CellHarvestor (Skatron, cat # 11019). Wash cells 3x with 1.0-ml sterile distilled water. Note: all procedures are done using sterile techniques in a biological flow hood.

11. Place filters in Scintillation vials and add 1.5 mls of ScimSafe Plus 50% scintillation fluid (Fisher, cat # SX-25-5).

12. Measure the amount of radioactivity incorporated in the lymphocytes using a beta counter (Micromedic System Inc., TAURUS Automatic Liquid Scintillation Counter) for 1.0 minute.

13. Calculate averages and standard deviations for each drug and express results as:

\[
\text{% Inhibition} = \left[ \frac{1 - \text{Ave CPM of test drug}}{\text{Ave CPM of zero drug}} \right] \times 100 \\
\text{% Proliferation} = 100 - \text{% Inhibition}
\]

From the results of the calcineurin assay and the mixed lymphocyte reaction assay, it was found that cyclosporines that have been chemically substituted and/or deuterated at the amino acid 1 position can possess significant immunosuppressant activity.

Example 11

Other cyclosporine derivatives of the invention which have been prepared include the following:
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**[0100]** Drug Composition Formulation and Elicitation of Immunosuppression

**[0101]** Determination of the physicochemical, pharmacodynamic, toxicological and pharmacokinetic properties of the cyclosporine derivatives disclosed can be made using standard chemical and biological assays and through the use of mathematical modeling techniques which are known in the chemical and pharmacological/toxicological arts. The therapeutic utility and dosing regimen can be extrapolated from the results of such techniques and through the use of appropriate pharmacokinetic and/or pharmacodynamic models.

**[0102]** The compounds of this invention may be administered neat or with a pharmaceutical carrier to a warm blooded animal in need thereof. The pharmaceutical carrier may be solid or liquid.

**[0103]** This invention also relates to a method of treatment for patients suffering from immunoregulatory abnormalities involving the administration of the disclosed cyclosporines as the active constituent.

**[0104]** For the treatment of these conditions and diseases caused by immunoregularity, a deuterated cyclosporin may be administered orally, topically, parenterally, by inhalation spray or rectally in dosage unit formulations containing...
conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral, as used herein, includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques.

[0105] The pharmaceutical compositions containing the active ingredient may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, color agents and preserving agents in order to provide pharmaceutically elegant and palatable preparation. Tablets containing the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients may also be manufactured by known methods. The excipients used may be for example, (1) inert diluents such as calcium carbonate, lactose, calcium phosphate or sodium phosphate; (2) granulating and disintegrating agents such as corn starch, or alginic acid; (3) binding agents such as starch, gelatin or acacia, and (4) lubricating agents such as magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. They may also be coated by the techniques described in the U.S. Pat. Nos. 4,256,108; 4,160,452; and 4,265,874 to form osmotic therapeutic tablets for controlled release.

[0106] In some cases, formulations for oral use may be in the form of hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin. They may also be in the form of soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

[0107] Aqueous suspensions normally contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients may be

[0108] (1) suspending agents such as sodium carboxyethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia;

[0109] (2) dispersing or wetting agents which may be

[0110] (a) a naturally-occurring phosphatid such as lecithin,
[0111] (b) a condensation product of an alkylene oxide with a fatty acid, for example, polyoxyethylene stearate,
[0112] (c) a condensation product of ethylene oxide with a long chain aliphatic alcohol, for example, heptadecylpolyethyleneoxyoctanol,
[0113] (d) a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol such as polyethylene sorbitol monooleate, or

[0114] (e) a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol anhydride, for example polyoxyethylene sorbitan monooleate.

[0115] The aqueous suspensions may also contain one or more preservatives, for example, ethyl or n-propyl p-hydroxybenzoate; one or more coloring agents; one or more flavoring agents; and one or more sweetening agents such as sucrose, aspartame or saccharin.

[0116] Oily suspension may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an antioxidant such as ascorbic acid.

[0117] Dispersible powders and granules are suitable for the preparation of an aqueous suspension. They provide the active ingredient in admixture with a dispersing or wetting agent, a suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example, those sweetening, flavoring and coloring agents described above may also be present.

[0118] The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil such as olive oil or arachis oils, or a mineral oil such as liquid paraffin or a mixture thereof. Suitable emulsifying agents may be (1) naturally-occurring gums such as gum acacia and gum tragacanth, (2) naturally-occurring phosphatides such as soy bean and lecithin, (3) esters or partial esters derived from fatty acids and hexitol anhydrides, for example, sorbitan monooleate, (4) condensation products of said partial esters with ethylene oxide, for example, polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavoring agents.

[0119] Syrups and elixirs may be formulated with sweetening agents, for example, glycerol, propylene glycol, sorbitol, aspartame or sucrose. Such formulations may also contain a demulcent, a preservative and flavoring and coloring agents.

[0120] The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to known methods using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find uses in the preparation of injectables.

[0121] The disclosed cyclosporines may also be administered in the form of suppositories for rectal administration of
the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

[0122] For topical use, creams, ointments, jellies, solutions or suspensions, etc., containing the disclosed cyclosporines are employed.

[0123] Dosage levels of the order from about 0.05 mg to about 50 mg per kilogram of body weight per day are useful in the treatment of the above-indicated conditions (from about 2.5 mg to about 2.5 gms. per patient per day).

[0124] The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a formula-

![Chemical Structure](image)

wherein R is

(i) a deuterium or
(ii) a saturated or unsaturated straight or branched aliphatic carbon chain of from 1 to 16 carbon atoms and optionally containing one or more deuterium atoms or an ester, ketone or alcohol of said carbon chain and optionally containing one or more substituents selected from halogen, nitro, amino, amid, aromatic, and heterocyclic, or
(iii) an aromatic or heterocyclic group containing one or more deuterium atoms, or
(iv) a methyl group and

X, Y, and Z are hydrogen or deuterium provided that if R is methyl then at least one of X, Y or Z is deuterium and wherein R is an OH or acetate or other ester or is an O and together with a carbon adjacent to a double bond on amino acid 1 forms a heterocyclic ring.

1. A cyclosporine A derivative with isotopic or chemical substitution in the amino acid position selected from the group consisting of 1, 3 and 9 or combinations thereof.
2. A cyclosporine A derivative according to claim 1 wherein one or more hydrogen atoms in the amino acid position selected from the group consisting of 1, 3 and 9 or combinations thereof are substituted with a deuterium atom and wherein said cyclosporine A derivative is optionally chemically substituted at the amino acid 9 position.
3. The cyclosporin A derivative according to claim 1 represented by the formula I:
4. The cyclosporine A derivative of claim 3 wherein R is a saturated or unsaturated aliphatic carbon chain of from 2 to 3 carbons.

5. The cyclosporine A derivative of claim 3 wherein R is a saturated or unsaturated aliphatic carbon chain of from 2 to 3 carbons containing one or more deuterium atoms.

6. The cyclosporine A derivative of claim 3 wherein R is methyl.

7. The cyclosporine A derivative of claim 4 wherein X, Y and Z are hydrogen.

8. The cyclosporine A derivative of claim 1 represented by formula 5g:

9. The cyclosporine A derivative of claim 1 represented by formula 5e:

10. A pharmaceutical composition comprising the cyclosporine A derivative of claim 1 or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier.

11. The cyclosporine A derivative of claim 3 wherein X, Y and Z are H and R is a member selected from the group consisting of —D, —CHO, —CDO, —CD₂, —CH═CD—CD₂, —CD═CD—CD₂, —CD═CD═CD—CD₂, —CH═CH—CH═CD—CD₂, —CD═CH—CD═CD—CD₂, —CH═CH—CH═CD—CD₂, —CD═CH═CD—CD₂, —CD═CD—CD₂, —CD═CD—CD₂, —CD═CD—CD₂, —CH═CH—CH═CD—CD₂, and CH═CH—CH═CH—CD₂.

12. A method of producing immunosuppression comprising:

administering to an animal in need thereof, an effective amount of a cyclosporine A derivative according to claim 1.

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